Reference: *Biol. Bull.*, **150**: 279–285. (April, 1976)

TRANSPORTATION AND MAINTENANCE OF ADULT SQUID (*DORYTEUTHIS BLEEKERI*) FOR PHYSIOLOGICAL STUDIES

GEN MATSUMOTO[1]

Electrotechnical Laboratory, Tanashi, Tokyo, Japan

Almost all the important problems in nerve physiology have been successfully approached through the study of animals with particularly large nerve cells or fibers that are amenable to experimentation. Two nerve fibers in the nervous system of the squid are the largest; they are often called giant nerve fibers. Their diameters are between 400 and 800 μ. A single nerve fiber from the live squid is easily obtained. The important characteristics of a single nerve fiber have been found or ascertained in the squid giant axons (Rosenberg, 1973). Only the squid giant axons have clarified basic processes associated with nerve excitation and with nervous conduction (Tasaki, 1968; Hodgkin, 1964).

Squid experiments are usually limited both regionally to some marine laboratories and seasonally by availability of natural squid stocks (Arnold, Summers, and Gilbert, 1974) (in our case in Japan, it is usually from October through December). These limitations can be overcome if the squid could be transported from several fishing sites with different squid seasons to a well-equipped laboratory, and if the squid could be maintained in an aquarium in the laboratory. Transportation and maintenance of the squid is one of the basic requirements for any study of squid axons in any laboratory located far from the sea (Spotte, 1973).

Here, successful experiments on the transportation and maintenance along these directions are reported.

Materials and Methods

Source of squid

Squid used in the maintenance study and subsequent physiological experiments were all *Doryteuthis bleekeri,* purchased from commercial fishermen operating near Jogashima Island, Kanagawa prefecture. They were caught in a seasonal fishery (October through December) using Japanese squid jiggers of the so-called "single line" technique. Individual jiggers are elongated, colored plastic lures about 9.0 cm long, with two circles of barbless hooks around the lower end. Ten to fifteen squid were purchased weekly in 1973 and 1974, resulting in a cumulative total of 292 experimental animals. Ambient conditions on the fishing grounds were measured on November, 1973; with the following results: depth of squid, 100 m or less; water temperature, 13° C; dissolved oxygen, 4.11 ml/l.

[1] Present address: National Institute of Mental Health, 9000 Rockville Pike, Bethesda, Maryland 20014.

Transportation

Squid were transported by truck using one of two kinds of containers. Initially (October and November, 1973), 40 cm diameter × 1m bags made of vinyl chloride were used. The bag was half-filled with filtered sea water, and two or three squid were gently put into it. The bag was then inflated with high pressure oxygen gas and put into a corrugated cardboard box. This is similar to the method used to transport live carp or goldfish, and is especially suitable for small numbers of squid.

After November 1973, an alternative mass transportation method was adopted, which was preferable since enough sea water to fill our aquarium could be obtained at the same time. Filtered sea water was taken from the Kanagawa Prefectural Fisheries Experimental Station at Jogashima Island before the squid purchase. A tank composed of a 1m × 1m × 1m skeleton box and dark green vinyl cloth carried both sea water and ten to fifteen squid. It was thought that the soft configuration might mitigate damage to the squid as a result of collisons with the tank walls. In transport, the tank was filled to the top to prevent splashing and any resultant stimulation of the squid. Oxygen gas was continuously bubbled into the tank from a high pressure source. The temperature of the sea water in the tank was 17 to 19° C and was not controlled. After a three to five hour trip, the squid were quickly transferred into a prepared aquarium. The trip was made once a week on an average, and the total number of trips in 1974 was 12. No deaths during the transport of the squid were observed.

Maintenance system

The maintenance system was located in a small wooden house approximately 3.2 m long by 3.8 m wide, especially built for this study. The windows were covered with black papers so that no direct sunlight could shine into the room, and it was kept in almost total darkness at all times when squid were present, which made the environment resemble the squid's natural habitat about 100 m from the sea surface.

The aquarium system consisted of a circular tank with lid, three filtering stages, an activated carbon filter, temperature control, air bubbler, and circulation pump (see Figure 1a). The outlet of the filtered, circulating sea water was directed so that it would flow peripherally around the circular aquarium (see Figure 1b). The flow velocity of circulating sea water was 5 m/min. This flow pattern may decrease squid collisions with the aquarium walls, since they appear to align themselves with the direction of flow. In 1975, a net pattern was drawn with black-colored vinyl paint on both inner and outer aquarium walls. This, in addition to the circulation of the sea water, completely prevented the squid from even touching the walls. Both outer and inner walls of the aquarium were made from polyethylene. The outer diameter was 1.5 m and the inner diameter was 0.5 m; the depth was 1.0 m. The lid satisfactorily prevented squid from getting out of the aquarium.

Each filtering stage was composed of layers of small stone, glass wool, activated charcoal, and several sizes of sand (Ooiso sands), as shown in Figure 1a. The area of filter 10A was 50 cm × 100 cm; depth was 45 cm. It was composed of two sizes of sand: for one the diameter was 0.5 mm, and for the other 1 mm, on an average. The thickness of each layer was 15 cm for coarser size of sand and 25 cm for finer one. The coarser size of sand was under the finer.

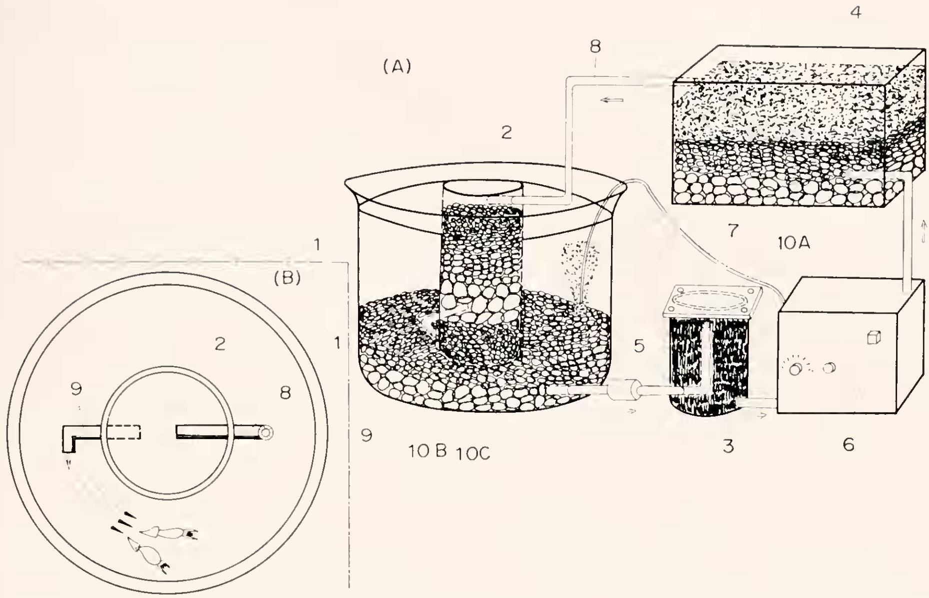

FIGURE 1. (a.) Configuration of the aquarium system. 1, shows the aquarium; 2, central cylinder which prevents squid movement in a radial direction and also serves as a container for filtering stage 10C; 3, activated charcoal filter; 4, filtering stage 10A; 5, dirt trap; 6, control box for circulation pump and temperature control unit; 7, air bubbler; 8, overflow from filter 10C; 9, seawater outlet; 10A, 10B, and 10C, three filtering stages. (b.) Circulation pattern of sea water in the aquarium, plan view.

Filter 10B was composed of layers of small stones (diameter of 5 mm, and thickness of 5 cm); glass wool (thickness of 5 mm when not wet); and sand of grain diameter 2 mm (thickness, 25 cm). The area of filter 10B was 1.5 m^2.

Filter 10C was composed of layers of small stones (diameter of 5 mm, and thickness, 10 cm); glass wool (thickness, 5 mm); activated charcoal (cylindrical pieces with diameters of 2.5 mm, and length of 4 mm; thickness of the layer, 20 cm); and sand (diameter of 2 mm on an average, and thickness, 40 cm). A layer of commercially available Zeolite (diameter of 4 mm and thickness, 5 cm) was added in 1975 to the above consituents of filter 10° C to moderate pH change.

The temperature control unit can regulate the sea water temperature in the aquarium at specific temperatures in the range 10° C and 25° C with an accuracy of ± 0.2° C. The circulation pump circulated the sea water at the rate of 200 l/min for the system (Spotte, 1973; Mariscal, 1974).

RESULTS

The heavy solid line in Figure 2 shows a typical percentage of survival in relation to number of maintenance days, obtained from a total of twenty squid in 1974. In 1974, undisturbed observations on the maintained squid were made on two sets of seven days, the initial week and the last week of the squid season. For both trials, one squid out of the ten maintained was dead on the seventh day. The remaining squid were used for our subsequent physiological experiments on the methods of observation. Therefore, an average survival percentage could not be obtained accurately, although the total number of the squid we used in 1974 was 192. For the first trial in 1975, the observation period was increased to three weeks without disturbance. For this trial, slices of raw tuna of approximately 300 g in total weight were given to 12 squid for three days as food. No natural death occurred during the three weeks except that one squid devoured another. In all other cases, the observation period was limited to one week because in the subsequent physiological experiments with squid giant axons, a consistent supply of eight to twelve live squid per week was found to be sufficient. Furthermore, many difficult problems associated with longer periods of squid maintenance were anticipated, such as regulation of sea water pH and salinity. For the present purpose of physiological studies, survival rates of 90% or better were satisfactory. Accordingly, the holding capacity of the aquarium system was designed originally to maintain ten to fifteen squid, and weekly purchases were anticipated.

Loss of squid during transportation did not occur, even though the fishing method and truck equipment were relatively crude operations. These conditions excited and/or tired the squid, because they appeared less vigorous and were alarmed by slight changes in their surroundings. For example, they would try to escape from 3- to 4 cm goldfish placed in the aquarium for food within the first day after arrival in the laboratory; some even inked under these circumstances. Squid would take goldfish on the second day, although we only repeated attempts to feed them on two occasions. At about the same time, the squid appeared much more accustomed to the aquarium environment and had recovered from their lack of vigor. Mating and spawning in the aquarium were often observed.

The aquarium system was usually preset to maintain a sea water temperature of 15° C, and the air bubbler was regulated to hold the dissolved oxygen level at about 4.1 ml/l. Temperature effects the survival mainly through associated changes in the dissolved oxygen concentration. On one occasion, almost all of the squid in the aquarium were moribund three hours after the air bubbler stopped.

Filtering capacity was the most important factor in the successful maintenance of squid. A single filter stage was used before the 1974 season (filter 10A, as shown in Figure 1). Survival of squid under these conditions is shown by the dashed line in Figure 2; half of the squid died in about one day. At the beginning of the squid season in 1974, filter 10B was added (see Figure 1), with no appreciable difference in survival rate over the previous experience. Soon thereafter, filter 10C was added, and a considerable improvement in survival was observed. That is, no natural death occurred within the first four days after stocking the aquarium and only 8% died within the first week from the 192 squid total. A typical survival pattern is shown as the heavy solid line in Figure 2. Change in survival is considered to be a discontinuous function of filtering capacity, as suggested by these results.

The conditions provided by the aquarium made collisions with the tanks walls infrequent. The fact that the squid oriented to the imposed circular flow was readily confirmed by reversing the outlet (number 9 in Figure 1) and observing

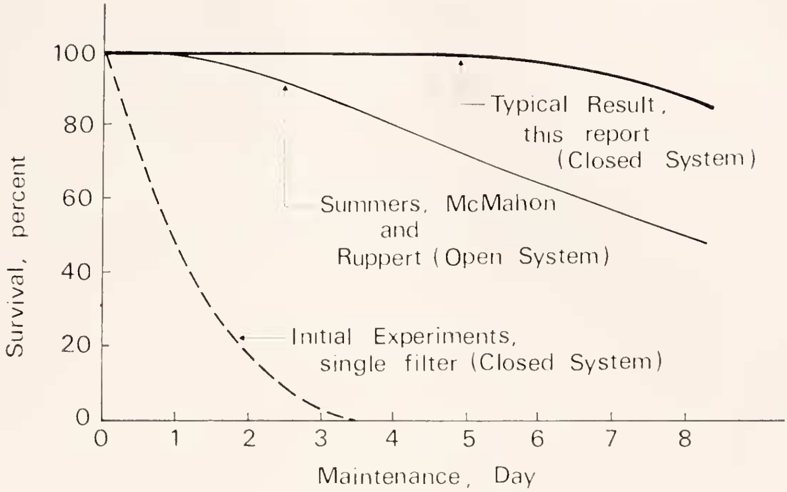

FIGURE 2. Squid survival over an eight day period. The heavy solid line shows experimental results for the system shown in Figure 1. The dashed line corresponds to the squid survival with the initial system before the addition of filtering stages 10B and 10C. The fine solid line shows results reported by Summers, McMahon and Ruppert (1974) using an open system and a bumper inside the tank walls.

that the squid quickly changed their direction of swimming. It was concluded in the initial stages of work in 1973 that avoidance of collision would not be a crucial factor for survival of squid, and efforts were concentrated on improving the filter capacity.

There was no appreciable difference in survival when the sea water temperature was varied over the range from 13° C to 20° C if enough oxygen was dissolved in the sea water, as was observed by Summers and McMahon (1970, 1974). However, there seemed to be a general tendency for spawning to occur at temperatures lower than 15° C.

Discussion

Systematic work on the maintenance of squid has been extensively reported by Summers and McMahon (1974) and Summers, McMahon and Ruppert (1974) on the northwest Atlantic squid, *Loligo pealei*. They concluded that observed improvements in survivorship of squid were due to modifications in the capture techniques, sorting of squid, and the introduction of a bumper system. Their median survival time (when survival becomes 50%) was eight days; their data are shown in Figure 2. They used an open system, where running sea water fully replaced the tank volume 1.33 to 2 times per hour. Their bumper system was composed of polyethylene tubing suspended inside the walls of rectangular tanks (Summers, McMahon and Ruppert, 1974), which absorbed the impact of squid collision with the barrier. Their method of capturing squid differed from the one described in this report.

Additional filtering stages were effective in maintaining a viable sea water quality and promoting longer squid survival. The degree of the improvement is difficult to determine quantitatively. It can be concluded from the experimental results that there is a critical amount of filtration necessary for the maintenance of the squid; in other words, a threshold level exists. This threshold correlates to the squid's anatomical structure. The gills are directly exposed to ambient sea water and may become fouled by particulate matter in the water through normal activity or respiration. Fine dirt of glass wool in the gills of moribund individuals was often observed, using an optical microscope. The experimental results also correlate with the fact that the squid are found in clear sea water at depths up to nearly 100 m.

It is noted here that success in the maintenance of squid represents a single parameter solution. For more complete success, many other factors, such as pH control, salinity regulation, feeding, and associated sea water quality parameters, will likely be involved. From the experimental result in 1975, despite only one trial, for longer periods of observation than a week, it can be safely concluded that sufficient feeding is another important factor in maintaining squid for longer periods. After giving raw tuna to the squid for food, the sea water in the aquarium should be allowed to overflow to eliminate oil sheets on the surface of the sea water. Detailed studies for longer periods of maintenance are left for future work.

The author would like to express his cordial thanks to Dr. J. Shimada for his collaboration at the initial stage of this study and his sincere encouragements throughout it; to Dr. Konrad Lorenz for his sincere discussions and especially for his valuable suggestion that the squid might require plenty of food in view of their rapid growth to adult size in only one year; and to Professor W. C. Summers for his valuable discussions and his help in revising this manuscript. The author also thanks Mr. H. Shimizu for his continuous encouragement of this work.

Summary

Transportation and maintenance of adult squid (*Doryteuthis bleekeri*) was investigated successfully. Squid were transported on a three to five hour trip and could then be maintained in circulating sea water in a closed system aquarium for up to three weeks. The most crucial factor for maintenance of squid was filtration of the sea water. The survival percentage of squid was 90–100% over the first week.

LITERATURE CITED

Arnold, J. M., W. C. Summers, and A. L. Gilbert, 1974. A guide to the laboratory use of the squid *Loligo pealei*. Marine Biological Laboratory ,Woods Hole, Massachusetts, 74 pp.

Hodgkin, A. L., 1964. *The conduction of the nervous impulse*. Liverpool University Press, Liverpool, England. pp. 20–29.

Tasaki, I. 1968. *Nerve excitation*. Charles C Thomas, Springfield, Illinois, pp. 20–26.

Mariscal, R. N., 1974. *Experimental marine biology.* Academic Press, New York, 373 pp.
Rosenberg, P., 1973. The giant axon of the squid: A useful preparation for neurophysiological and pharmacological studies. F. Reiner, Ed., *Methods of neurochemistry, volume IV.* Marcel Dekker, New York, 327 pp.
Spotte, S. H., 1973. *Marine aquarium keeping: the science, animals,* and *art.* Wiley-Interscience, New York, 171 pp.
Summers, W. C., and J. J. McMahon, 1970. Survival of unfed squid, *Loligo pealei,* in an aquarium. *Biol. Bull.*, **138**: 389–396.
Summers, W. C., and J. J. McMahon, 1974. Studies on the maintenance of adult squid (*Loligo pealei*). I. Factorial survey. *Biol. Bull.*, **146**: 279–290.
Summers, W. C., J. J. McMahon, and G. N. P. A. Ruppert, 1974. Studies on the maintenance of adult squid (*Loligo pealei*). II. Empirical extensions. *Biol. Bull.* **146**: 291–302.